MICRO-TUMOR BREAST PRIMARY CANCER TISSUES : ONCOKIT

Cost effective

Fast, efficient, and cost-effective. Test many conditions in less time.

Ethical

All human samples are collected from informed donors with ethical consent, following international standards.

Scientific

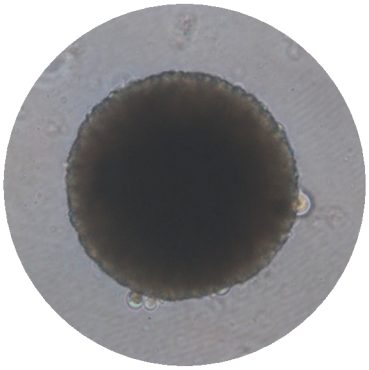
Advanced 2D, 3D, and Ex-vivo technologies for high reproducibility and environment control.

Context

The development of a new drug takes over ten years, costs around one billion euros, and fails in over 90% of cases, mainly due to the lack of reliable and predictive preclinical models.

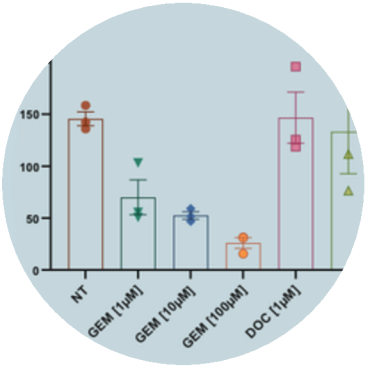
Our models

Our **patient-derived 3D tumor models** faithfully reproduce key tumor features, including **proliferation**, **necrosis**, and **biomarker expression**. They enable multi-week monitoring of drug penetration, efficacy, and resistance. Compatible with **chemotherapies**, **targeted therapies**, and **immunotherapies**, these models provide a **standardized** and **reproducible system**, ideal for rapid **preclinical treatment selection**.



Cancer micro-tumor

Application



Readouts

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CONTEXT

The development of a **new drug** takes over ten years, costs around **one billion euros**, and **fails in over 90% of cases**, mainly due to the **lack of reliable and predictive preclinical models.**

Animal models often translate poorly to humans, and while **micro-tumor** represent a major advance, they were initially created from homogeneous cell lines where self-aggregation is simple, unlike with **primary human tissues**. Testing **advanced chemotherapeutic strategies** thus requires primary cancer models.

We therefore investigated **primary epithelial breast (BRE) cancer** for cohesive, reproducible micro-tumor structures. Having established protocols for harvesting and cryopreserving primary cancer cells and associated fibroblasts, we assessed micro-tumor reproducibility using ethically biobanked breast and patient-derived tumor cells in ultralow-attachment (ULA) conditions

MATERIALS & METHODS

Spheroids were formed by seeding 3,000 cells per well in ultralow-attachment (ULA) plates and maintained in culture for 10 days. After the formation and culture period, spheroids were exposed for 48 hours to two chemotherapeutic agents, Gemcitabine (1 µM, 10 µM, 100 µM) and Docetaxel (1 µM, 10 µM, 100 µM). Following treatments, spheroid metabolic activity and diameter were quantified. Cell viability was assessed using the LIVE/DEAD™viability/cytotoxicity kit, while proliferation, apoptosis, and hypoxia were evaluated through immunostaining of Ki67, Caspase 3/7, and hypoxic markers, respectively.

RESULTS

Metabolic activity

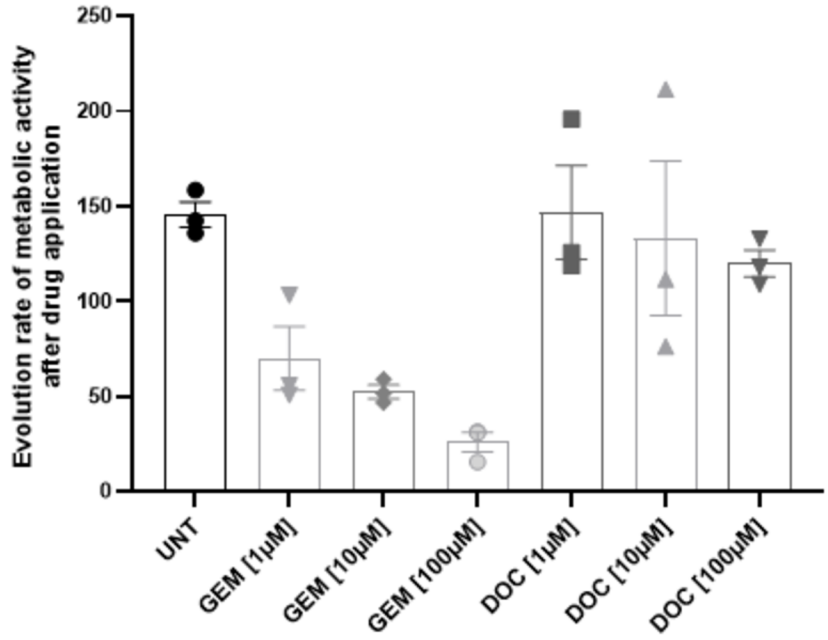

***Figure 1**: Evolution rate of metabolic activity of spheroids after 48h exposure of 2 chemotherapeutic treatments (UNT = Untreated, GEM = Gemcitabine, DOC= Docetaxel)*

After 48 hours of treatment, both Gemcitabine and Docetaxel induced a clear, dose-dependent reduction in metabolic activity compared to non-treated (uNT) spheroids. The strongest decrease was observed at the highest concentrations (100 µM). Lower concentrations (1 µM and 10 µM) resulted in a moderate but consistent decline, confirming aconcentration-dependent cytotoxic response (**Figure 1**)

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SPHEROIDS SIZE AND MORPHOLOGY

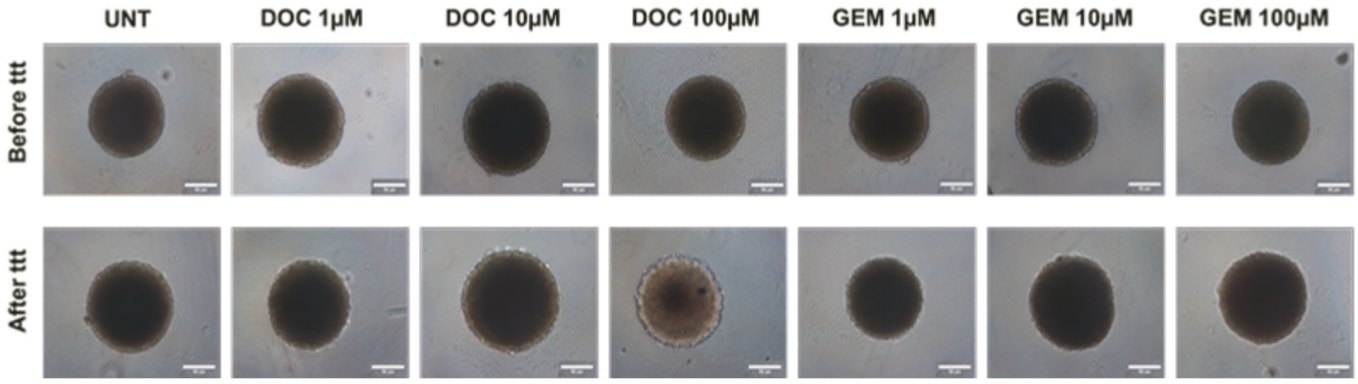

***Figure 2**: Microscopic pictures of tumoroids (seeding : 3,000 cells/well) after 48hrs of chemotherapeutic treatment (ttt = treatment, UNT = Untreated, GEM = Gemcitabine, DOC = Docetaxel) Scale bar = 50µm.*

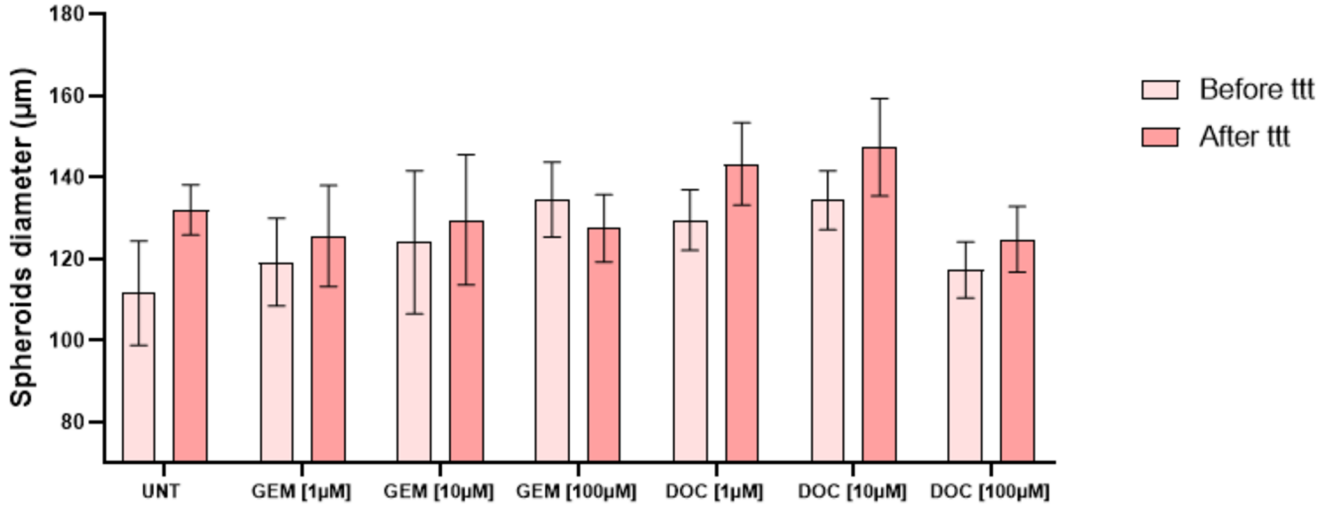

***Figure 3**: Comparison of spheroid diameters (µm) before and after 48hrs of chemotherapeutic treatment (UNT= Untreated, GEM = Gemcitabine, DOC = Docetaxel).*

BRE cells formed highly cohesive micro-tumor with clear 3D architecture, consistent with theexpected morphology of this cancer type and proportional to the initial seeding density **(Figure2)**.

After 48hours of treatment with Gemcitabine or Docetaxel at all tested concentrations, no significant change in spheroid diameter was observe dindicating that overall spheroid structure remained stable despite treatment-induced metabolic alterations. BRE spheroids exhibited a uniform and compact morphology under all conditions **(Figure 3)**.

LIVE/DEAD, PROLIFERATION, APOPTOSIS, AND HYPOXIA

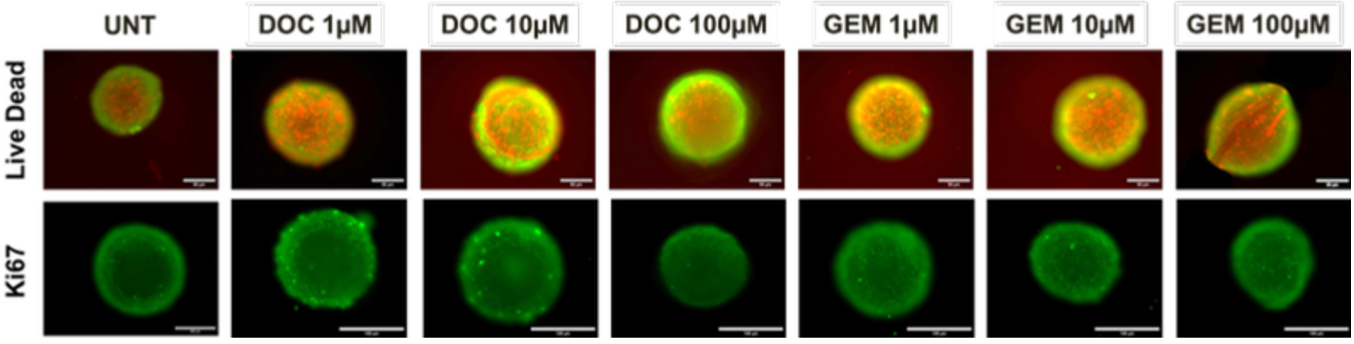

***Figure 4**: Evaluation of spheroid viability and proliferation after 48hrs of chemotherapeutic treatment. (UNT = Non treated, GEM = Gemcitabine, DOC = Docetaxel) (Live= Green, Dead =Red/ Ki67 = Green). Live Dead Scale bar=50µm; Ki67 Scale bar=100µm*

Cell viability was assessed using the LIVE/DEAD™viability/cytotoxicity kit with the same acquisition parameters for all the conditions. Untreated (UNT) spheroids exhibited predominantly green fluorescence, indicating high viability and structural integrity. In contrast,spheroids treated with Gemcitabine or Docetaxel showed a progressive increase in red fluorescence with increasing drug concentrations, reflecting a concentration-dependent rise in cell death (**Figure 4**). Cell proliferation was analysed through Ki67 immunostaining. Ki67 staining revealed a proliferative signal in controls that decreased progressively with treatment,indicating reduced proliferative activity following chemotherapeutic exposure **(Figure 4)**.

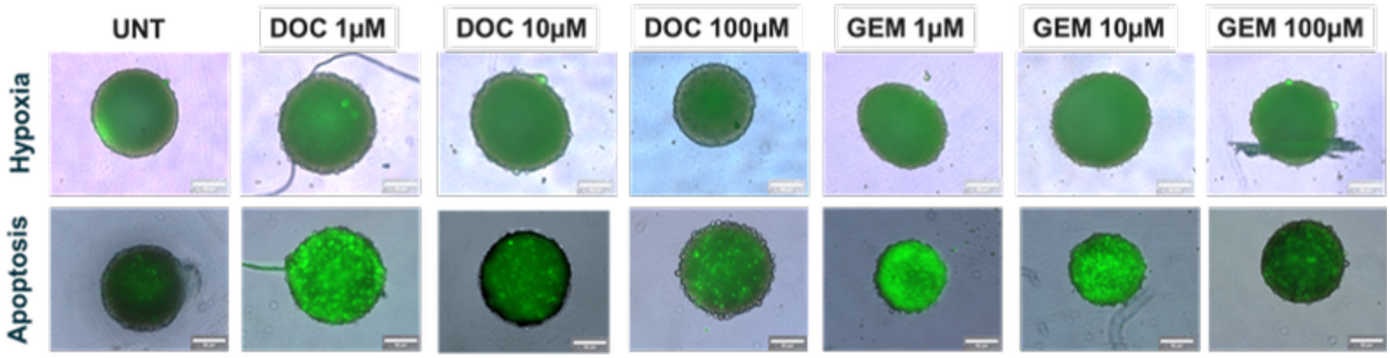

***Figure 5:** Evaluation of spheroid, hypoxia, and apoptosis after 48hrs of chemotherapeutic treatment. (UNT = Untreated, GEM = Gemcitabine, DOC = Docetaxel) (Hypoxia = Green / Apoptosis = Green) Scale bar 50µm*

Hypoxia was visualized using a fluorescent hypoxia probe (Image-iT™Green HypoxiaReagent). All spheroids exhibited hypoxic core, consistent with their 3D structure and size (>200 µm). The hypoxia signal remained similar across all conditions, suggesting that 48-hours drug treatment did not markedly affect oxygen gradients or internal tissue organization **(Figure 5)**.

Apoptosis was evaluated usinga caspases detectionkit (Caspase-3/7 Detection Reagents). This marker detects active caspases and therefore labels apoptotic cells with green fluorescence. The fluorescence signal increased progressively with Gemcitabine and Docetaxel concentration, indicating enhanced caspase activation and apoptotic response, particularly at 100 µM **(Figure 5)**.

Overall, chemotherapeutic treatment reduced viability and proliferation while enhancing apoptosis, yet spheroid integrity and hypoxic organization remained largely preserved.

✓ CONCLUSION

Our models reproduce the **main histological features** of patient tumors, including **proliferative areas**, **necrotic cores**, and the e**xpression of relevant biomarkers**. Drug penetration, efficacy, and resistance can be monitored over several weeks, enabling the evaluation of different therapeutic classes such as **chemotherapeutics**, **targeted therapies**, or **immunotherapies**. These patient-derived 3D cancer models are **standardized** and **reproducible systems**.

By **preserving patient-specific tumor characteristics** and **testing standard chemotherapies**, they allow for patient variability and **facilitate preclinical treatment selection**. This **increases their clinical relevance** and positions them a useful link between *in vitro* screening and *in vivo* validation.